

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Group Art Unit: Not yet assigned

LAZZARINI et al.

Serial No.: Not yet assigned

Filed: Submitted herewith

**For: ANTIBIOTIC 107891, ITS FACTORS
A1 AND A2, PHARMACEUTICALLY
ACCEPTABLE SALTS AND
COMPOSITIONS, AND USE
THEREOF**

PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Before the calculation of claim fees, please amend the subject application as follows.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims begin on page 3 of this paper.

Remarks begin on page 11 of this paper.

CERTIFICATE OF MAILING (37 C.F.R. §1.10)

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January 11, 2005
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AMENDMENTS TO THE SPECIFICATION

Please insert the following paragraph before the paragraph beginning on page 1, line 3.

-- This is a national stage application of international application PCT/EP2004/007658, filed July 12, 2004, which in turn claims priority to European Application No. 03016306.7, filed July 18, 2003. All of the above applications are expressly incorporated herein by reference. -

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1-23. (Canceled)

24. (New) Antibiotic 107891 complex comprising Factor A1 and Factor A2 being a white powder having the following characteristics:

(A) Mass spectrum recorded from a 0.2 mg/ml solution in methanol:water 80/20 (v/v) with trifluoracetic acid 0.1% on a ThermoFinnigan LCQ Deca instrument fitted with an electrospray source, using ThermoFinnigan calibration mix under the following electrospray conditions: spray voltage: 4.7 kV; capillary temperature: 220° C; capillary voltage: 3V; infusion mode 10 μ l/min, showing two double protonated ions at m/z 1124 and m/z 1116, corresponding to the lowest isotope composition of Factor A1 and A2, respectively;

(B) Infrared spectrum recorded in KBr with a Bruker FT-IR spectrophotometer model IFS 48, exhibiting absorption maxima at (cm^{-1}): 3263; 2929; 1661; 1533; 1402; 1114; 1026;

(C) U.V. spectrum performed in methanol: H_2O 80:20 (v/v) with a Perkin-Elmer spectrophotometer Lambda 16, exhibiting two shoulders at 226 and 267 nm;

(D) ^1H -NMR spectrum recorded at 600 MHz in the mixture methanol-d4: H_2O (pH 4.3 HCl) 40:10 (v/v) at 40°C on a Bruker AMX 600 spectrometer applying a water suppression sequence using as internal standard the residual signal of methanol-d4 at 3.31 ppm, exhibiting the following signals [δ =ppm multiplicity; (attribution)]: 0.93 d (CH_3), 0.98 d (CH_3), 1.07 t (overlapped CH_3 's), 1.18 t (overlapped CH_3 's), 1.26 s (CH_3), 1.30 t (overlapped CH_3 's), 1.62-1.74 m (CH_2), 1.78 d (CH_3), 1.80 d (CH_3), 2.03 m (CH_2), 2.24 m (CH), 2.36 m (CH_2), 2.72-3.8 m (peptidic alpha CH's), 3.8-5.2 m (peptidic alpha CH's), 5.53-6.08 s (CH_2), 5.62 d (CH double bond), 6.42 m (CH), 6.92 d (CH double bond), 7.0-7.55 m (aromatic CH's), 7.62-10.4 d and m (aromatic and peptidic NH's);

(E) ^{13}C -NMR spectrum recorded in the mixture methanol-d4: H_2O (pH 4.3 HCl) 40:10 (v/v) at 40°C on a Bruker AMX 600 spectrometer, using as internal standard the

residual signal of methanol-d4 at 49.15 ppm, exhibiting the following signals: [δ =ppm; (attribution)]: 13.6-23.2 (aliphatic CH₃'s), 26.16-73 (aliphatic CH₂'s and peptidic alpha CH's), 105-136 (aromatic and double bonds CH's and quartenary carbons), 164.3-176.3 (peptidic carbonyls);

(F) the acid hydrolysate in 6N HCl, (105°C, 24 h) showing the presence of the following amino acids, along with other unidentified peaks, after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate: lanthionine, methyllanthionine, glycine, proline, valine, aspartic acid (hydrolysis product of asparagine), phenylalanine and leucine;

(G) the acid hydrolysate in 4N methanesulfonic acid containing 0.2% (w/v) 3-(2-aminoethyl) indole as catalyst (115°C, 16h) showing the presence of 5-chlorotryptophan; and

(H) a basic ionizable function detected by acid/base titration performed with 0.01 N potassium hydroxide in 2-methoxyethanol (MCS):H₂O 12:3 (v/v) containing a molar excess of 0.01 N hydrochloric acid.

25. (New) Antibiotic 107891 Factor A1 being a white powder having the following characteristics:

(A) a doubly protonated ion at m/z 1124 corresponding to the lowest isotope composition in mass spectrum recorded from a 0.1 mg/ml solution in acetonitrile:water 50:50 (v/v) with acetic acid 0.5% on a ThermoFinnigan LCQ Deca instrument fitted with an electrospray source, using ThermoFinnigan calibration mix under the following electrospray conditions: spray voltage: 4.7 kV; capillary temperature: 250° C; capillary voltage: 8V; infusion mode 10 μ l/min;

(B) the exact mass of antibiotic determined by using a Bruker Daltonics APEX II, 4.7 Tesla spectrometer fitted with an electrospray source, corresponding to a molecular weight of 2246.71 \pm 0.06, calculated monoisotopic mass from [M+2H]²⁺ at m/z 1124.36124 (accuracy 30 ppm);

(C) when dissolved in CD₃CN:D₂O (1:1), ¹H NMR spectrum exhibiting the following groups of signals (in ppm) at 600 MHz using CD₃CN as internal standard (1.94 ppm), [δ =ppm, multiplicity; (attribution)]: 0.84 d (CH₃), 0.89 d (CH₃), 0.94 t (overlapped

CH₃'s), 1.1 d (CH₃), 1.13 d (CH₃), 1.15 t (overlapped CH₃'s), 149 m (CH₂), 1.69 d (CH₃), 1.75 m (CH₂), 2.11 m (CH), 2.26 m (CH), 2.5 m (CH₂), 2.68 – 3.8 m (peptidic CH_β's), 3.8 – 5.0 m (peptidic CH_α's), 5.45 – 6.17 s (CH₂), 5.58 d (CH double bond), 6.36 m (CH), 6.86 d (CH double bond), 7.0 – 7.45 m aromatic CH's);

(D) when dissolved in CD₃CN:D₂O (1:1), ¹³C NMR spectrum exhibiting the following signals (in ppm) at 600 MHz using CD₃CN as internal standard (1.39 ppm), [δ=ppm; (attribution)]: 13.6 – 23.03 (aliphatic CH₃'s), 25.69 – 77.9 (aliphatic CH₂'s and peptidic CH_α's), 105 – 137.3 (aromatic and double bonds CH's and quaternary carbons), 165.6 – 176.6 (peptidic carbonyls);

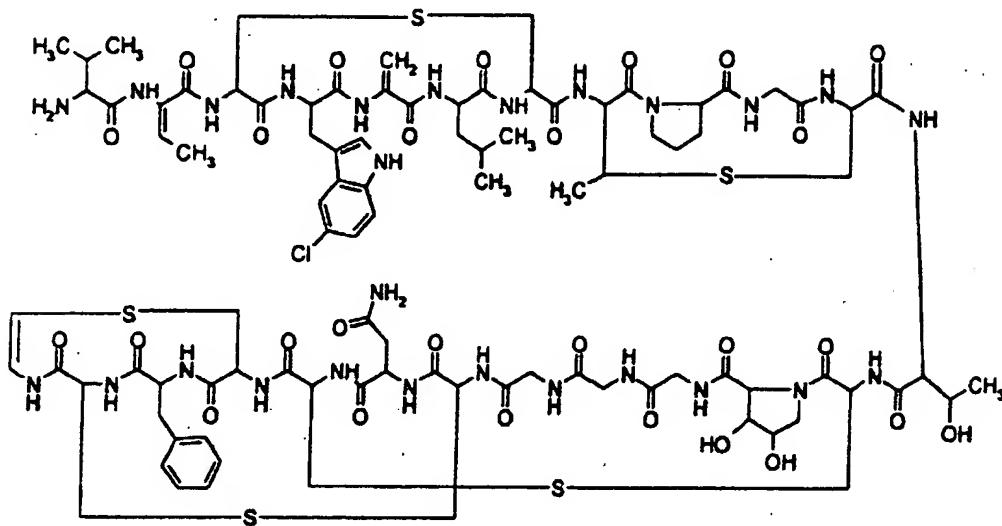
(E) Infrared spectrum recorded in KBr with a Bruker FT-IR spectrophotometer model IFS 48 exhibiting absorption maxima at (cm⁻¹): 3294; 3059; 2926; 1661; 1529; 1433; 1407; 1287; 1114; 1021;

(F) U.V. spectrum recorded in methanol:H₂O (in ratio 80:20) with a Perkin-Elmer spectrophotometer Lambda 16 exhibiting two shoulders at 226 and 267 nm;

(G) The acid hydrolysate in 6N HCl, (105°C, 24 h) showing the presence of the following amino acids, along with other unidentified peaks, after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate: lanthionine, methyllanthionine, glycine, proline, valine, aspartic acid (hydrolysis product of asparagine), phenylalanine, and leucine; and

(H) the acid hydrolysate in 4N methanesulfonic acid containing 0,2% (w/v) 3-(2-aminoethyl)indole as catalyst (115°C, 16h) showing the presence of 5-chlorotryptophan.

26. (New) The antibiotic 107891 Factor A1 of claim 25, wherein the antibiotic 107891 Factor A1 has the following structural formula:



27. (New) Antibiotic 107891 Factor A2 being a white powder having the following characteristics:

- (A) a doubly protonated ion at m/z 1116 corresponding to the lowest isotope composition in mass spectrum recorded from a 0.1 mg/ml solution in acetonitrile:water 50:50 (v/v) with acetic acid 0.5% on a ThermoFinnigan LCQ Deca instrument fitted with an electrospray source, using ThermoFinnigan calibration mix under the following electrospray conditions: spray voltage: 4.7 kV; capillary temperature: 250° C; capillary voltage: 8V; infusion mode 10 μ l/min;
- (B) the exact mass determined by using a Bruker Daltonics APEX II, 4.7 Tesla spectrometer fitted with an electrospray source, corresponding to a molecular weight of 2230.71 \pm 0.06, calculated monoisotopic mass from $[M+2H]^{2+}$ at m/z 1116.36260 (accuracy 30 ppm);
- (C) when dissolved in $CD_3CN:D_2O$ (1:1), 1H NMR spectrum exhibiting the following signals (in ppm) at 600 MHz using CD_3CN as internal standard (1.94 ppm),

[δ =ppm, multiplicity; (attribution)]: 0.84 d (CH_3), 0.88 d (CH_3), 0.94 d (CH_3), 1.06 d (CH_3), 1.14 d (CH_3), 148 m (CH_2), 1.65-1.75 m (CH_2), 1.67 d (CH_3), 2.15 m (CH), 2.25 m (CH), 2.5 m (CH_2), 2.77 – 3.8 m (peptidic CH_β 's), 3.8 – 4.9 m (peptidic CH_α 's), 5.45 – 6.14 s (CH_2), 5.59 d (CH double bond), 6.34 m (CH), 6.84 d (CH double bond), 7.0 – 7.42 m (aromatic CH 's);

(D) when dissolved in $\text{CD}_3\text{CN}:\text{D}_2\text{O}$ (1:1), ^{13}C NMR spectrum exhibiting the following signals (in ppm) at 600 MHz using CD_3CN as internal standard (1.39 ppm), [δ =ppm; (attribution)]: 13.6 – 22.9 (aliphatic CH_3 's), 25.65 – 73 (aliphatic CH_2 's and peptidic CH_α 's), 105 – 137.3 (aromatic and double bonds CH 's and quaternary carbons), 165.7 – 176.1 (peptidic carbonyls);

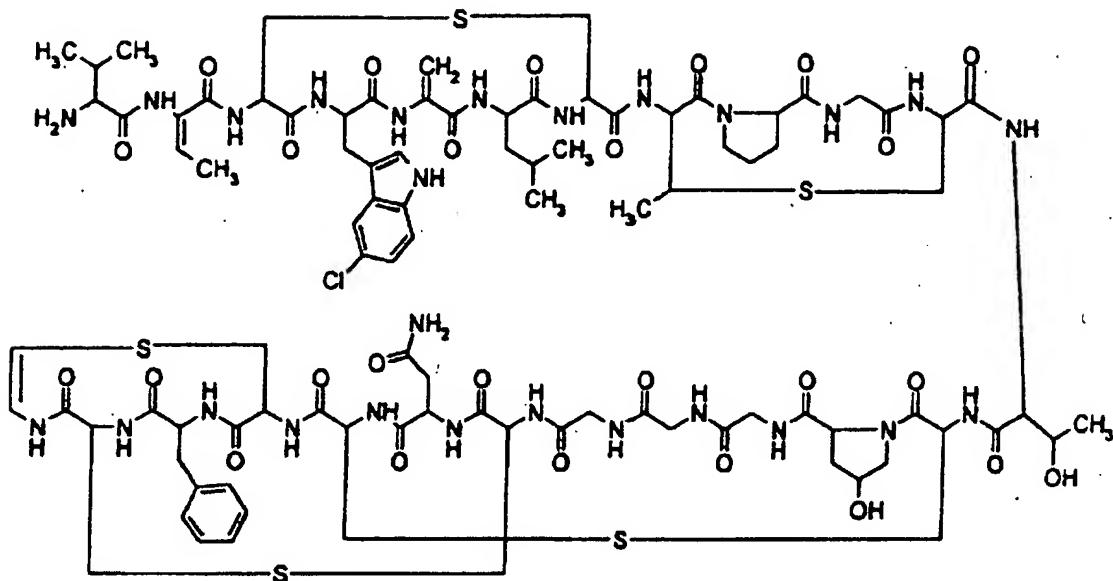
(E) infrared spectrum recorded in KBr with a Bruker FT-IR spectrophotometer model IFS 48, exhibiting absorption maxima at (cm^{-1}): 3296; 3060; 2928; 1661; 1529; 1433; 1407; 1288; 1116;

(F) U.V. spectrum recorded in methanol: H_2O (in ratio 80:20) with a Perkin-Elmer spectrophotometer Lambda 16 exhibiting two shoulders at 226 and 267 nm;

(G) the acid hydrolysate in 6N HCl, (105°C, 24 h) showing the presence of the following amino acids, along with other unidentified peaks, after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate: lanthionine, methyllanthionine, glycine, proline, valine, aspartic acid (hydrolysis product of asparagine), phenylalanine and leucine; and

(H) the acid hydrolysate in 4N methanesulfonic acid containing 0.2% (w/v) 3-(2-aminoethyl)indole as catalyst (115°C, 16h) showing the presence 5-chlorotryptophan.

28. (New) The antibiotic 107891 Factor A2 of claim 27, wherein the antibiotic 107891 Factor A2 has the following structural formula:



29. (New) A process for producing antibiotic 107891 and its Factors A1 and A2 and the salts thereof, comprising the steps of:

cultivating *Microbispora* sp. ATCC PTA-5024 or a variant or mutant thereof maintaining the ability to produce said antibiotic, under aerobic conditions, in an aqueous nutrient medium containing an assimilable source of carbon, nitrogen and inorganic salts; isolating the resulting antibiotic from the mycelium and/or the filtered fermentation broth; and

purifying the isolated antibiotic 107891.

30. (New) The process according to claim 29, wherein the strain *Microbispora* sp. ATCC PTA-5024 or the antibiotic 107891 producing a variant or mutant thereof are pre-cultured.

31. (New) The process according to claim 29, wherein the isolation of the antibiotic 107891 is carried out by filtering the fermentation broth and the antibiotic is recovered from the filtered fermentation broth according to a technique selected from the group consisting of extraction

with a water-immiscible solvent, precipitation by adding a non-solvent or by changing the pH of the solution, absorption chromatography, partition chromatography, reverse phase partition chromatography, ion exchange chromatography, molecular exclusion chromatography, and a combination of two or more of said techniques.

32. (New) The process according to claim 29, wherein the isolation of the antibiotic 107891 is carried out by separating the mycelium from the supernatant of the fermentation broth and the mycelium is extracted with a water-miscible solvent whereby, after the removal of the spent mycelium, a water-miscible solution containing the crude antibiotic is obtained, which can be processed either separately or in pool with the filtered fermentation broth to recover the antibiotic 107891 by means of a technique selected from the group consisting of extraction with a solvent, precipitation by adding a non-solvent or by changing the pH of the solution, absorption chromatography, partition chromatography, reverse phase partition chromatography, ion exchange chromatography and molecular exclusion chromatography, and a combination of two or more of said techniques.

33. (New) The process according to claim 32, wherein the concentration of the water-miscible solvent in the mycelium extract is reduced before it is processed to recover the antibiotic therefrom.

34. (New) The process according to claim 31, wherein the filtered fermentation broth is contacted with an absorption resin, and said resin is eluted with a polar, water-miscible solvent or a mixture thereof with water, whereby a solution containing the crude antibiotic 107981 is obtained.

35. (New) The process according to claim 34, wherein the absorption resin is selected from the group consisting of a polystyrene, a mixed polystyrene-divinylbenzene, and a polyamide resin.

36. (New) The process according to claim 32, wherein the mycelium is extracted with a C₁-C₃ alkanol, and the mycelium extract is contacted with an absorption resin, and eluted therefrom with a polar water-miscible solvent or a mixture thereof with water, whereby a solution containing the crude antibiotic 107891 is obtained.

37. (New) The process according to claim 36, wherein the solutions containing the crude antibiotic 107891 are pooled and processed for further purification of said antibiotic 107891.

38. (New) The process according to claim 36, wherein the solution containing the crude antibiotic 107981 is concentrated and then freeze-dried to yield a crude antibiotic 107891 solid product.

39. (New) The process according to claim 34, wherein the absorption resins containing the absorbed antibiotic are pooled and their mixture is eluted with a polar, water-miscible solvent or a mixture thereof with water.

40. (New) The process according to claim 29, wherein the antibiotic 107981 is purified by means of a chromatographic procedure.

41. (New) The process according to claim 40, wherein the chromatographic procedure is selected from the group consisting of preparative HPLC and medium pressure chromatography.

42. (New) The process according to claim 29, wherein Factor A1 and Factor A2 are separated by preparative HPLC from the purified antibiotic 107891.

43. (New) A pharmaceutical composition comprising an antibiotic selected from antibiotic 107891, antibiotic 107891 Factor A1, antibiotic 107891 Factor A2, and a mixture of said Factors in any proportion or a pharmaceutically acceptable salt thereof with an acid.

44. (New) The pharmaceutical composition according to claim 43, further comprising a pharmaceutically acceptable carrier.

REMARKS

Applicants request entry of the above amendments before examination, and before calculation of claim fees for the above-identified application. Claims 1-23 have been canceled. Claims 24-44 have been newly added. Support for these new claims can be found in the specification at, e.g., page 22, line 20 – page 35, line 2; page 45, line 7, page 51, line 6; and original claims 1-19. Therefore, no new matter has been added with these amendments.

Respectfully submitted,

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